Novel copper complexes as potential proteasome inhibitors for cancer treatment (Review)

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Abstract. The use of metal complexes in the pharmaceutical industry has recently increased and as a result, novel metal-based complexes have initiated an interest as potential anticancer agents. Copper (Cu), which is an essential trace element in all living organisms, is important in maintaining the function of numerous proteins and enzymes. It has recently been demonstrated that Cu complexes may be used as tumor-specific proteasome inhibitors and apoptosis inducers, by targeting the ubiquitin-proteasome pathway (UPP). Cu complexes have demonstrated promising results in preclinical studies. The UPP is important in controlling the expression, activity and location of various proteins. Therefore, selective proteasome inhibition and apoptotic induction in cancer cells have been regarded as potential anticancer strategies. The present short review discusses recent progress in the development of Cu complexes, including clioquinol, dithiocarbamates and Schiff bases, as proteasome inhibitors for cancer treatment. A discussion of recent research regarding the understanding of metal inhibitors based on Cu and ligand platforms is presented.

Contents

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Abbreviations: Cu, copper; DSF, disulfiram; PDTC, pyrrolidine dithiocarbomate; DDTC, diethyldithiocarbomate; CQ, clioquinol; 8-OHQ, 8-hydroxyquinoline; PHEN, 1,10-phenanthroline; PT, pyrithione, UPP, ubiquitin-proteasome pathway; CT, chymotrypsin; AR, androgen receptor; ALDH, aldehyde dehydrogenase

Key words: copper complex, proteasome inhibitor, ubiquitin-proteasome pathway, 8-hydroxyquinoline, clioquinol, dithiocarbamates, Schiff base, 1,10-phenanthroline

1. Introduction

Metal ions are required for the maintenance of numerous critical functions in living organisms and have been used to treat various human diseases (1-4). Platinum-based drugs, including cisplatin and carboplatin (Fig. 1) have been developed as effective anticancer drugs used in chemotherapy of various solid human tumors (5). In addition to platinum-based drugs, complexes of several other metals, including copper (Cu), zinc, gold, palladium and gallium, have demonstrated promising antitumor activities *in vitro* and *in vivo* in cancer therapy (6-9). The disulfiram (DSF)-Cu and clioquinol (CQ)-Cu complexes are being investigated as potential therapeutics for human cancer. Previous results have indicated that these complexes exhibit potent proteasome-inhibitory and apoptosis-inducing activities when the Cu was transported into cancer cells; however, they are minimally toxic toward normal cells (10-12). The use of metal complexes in the pharmaceutical industry is increasing, thus leading to the development of metal-based complexes as potential anticancer agents.

It has previously been demonstrated that the ubiquitin-proteasome pathway (UPP) is important in controlling the expression, activity and location of various cellular proteins (13). Ciechanover, Hershko and Rose were awarded the Nobel Prize in Chemistry in 2004 for its discovery (14,15). The UPP is responsible for ubiquitination and proteasomal degradation, which regulates cell cycle progression, signal transduction, differentiation, proliferation and apoptosis (16). Tagged proteins are degraded by the 26S proteasome, which is localized in the nucleus and cytosol of cells. The 26S proteasome is a multicatalytic enzyme complex, which consists of a 20S catalytic core and two 19S regulatory complexes (17-19). The 20S proteasome consists of four stacked heptameric ring structures, which are composed of two different types of subunits: α and β (20). The outer two rings in the stack each consist of seven α subunits, which allow unfolded

proteins to enter the 20S core, whereas β subunits in the inner two rings primarily contain three distinct catalytic subunits: β1, β2 and β5, which are responsible for caspase or peptidyl-glutamyl peptide-hydrolyzing-like, trypsin-like and chymotrypsin (CT)-like activity, respectively (21). Therefore, it has been suggested that proteasomal activity contributes to the pathological development of numerous diseases, including inflammation, neurodegeneration and cancer (22). Notably, it has previously been demonstrated that inhibition of the CT-like activity is associated with induction of tumor cell apoptosis programs (3,23,24).

Selective proteasome inhibition in cancer cells and induction of apoptosis have been regarded as potential anticancer strategies (25). Various proteasome inhibitors have been applied in preclinical experiments and clinical studies as novel anticancer agents (18). Bortezomib (Velcade; Fig. 1) and carfilzomib (Kyprolis; Fig. 1), to the best of our knowledge, are the only proteasome inhibitors that have been granted approval by the US Food and Drug Administration, in 2003 and 2012, respectively (26,27). Bortezomib is a reversible inhibitor of the 26S proteasome, which has been used clinically for the treatment of multiple myeloma (MM) and mantle cell lymphoma (28). Carfilzomib is a second-generation proteasome inhibitor, which is primarily used for the treatment of patients with MM following treatment with bortezomib and an immunomodulatory agent (29,30). Proteasome inhibitors have been demonstrated to be highly effective against several cancers in preclinical and clinical trials; however, resistance (2) and toxicity (31-33) have emerged as limiting factors of the continued clinical use of these drugs. A pharmaceutical aim for the future is to continue to develop a novel generation of proteasome inhibitors with far less toxicity and a broader spectrum of activity. Various metal complexes, including gold, Cu and zinc have been investigated as potential anticancer drugs, due to the success of platinum-based anticancer therapy. Metal-based complexes are currently under investigation due to their ability to exert potent antiproliferative effects by targeting the UPP, resulting in novel opportunities in cancer therapy (23). The present review focuses on recent advances in the development of metal complexes as proteasome inhibitors, with emphasis on Cu complexes for cancer treatment.

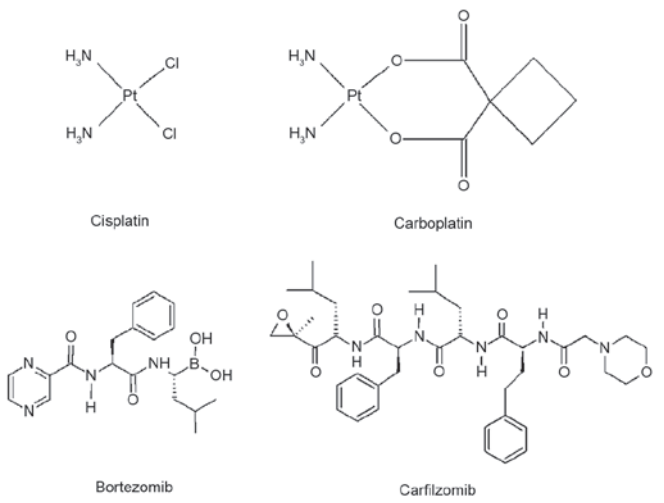

Figure 1. Chemical structures of cisplatin, carboplatin, bortezomib and carfilzomib.

2. Cu and Cu-based complexes

Cu is an essential trace element in all living organisms, which is important in the process of internal oxidation and reduction. All animals, including humans, require a finite amount of Cu for survival and normal physiological function. Cu is important in maintaining the correct functionality of proteins and enzymes. Numerous key enzymes require the participation and activation of Cu to affect metabolic processes in organisms (34,35). Cu levels in serum and tissue have been demonstrated to be significantly greater in various human tumors, including breast (36), prostate (37), colon (37), lung (38) and brain cancers (39). Kuo *et al* (36) reported that serum and tissue Cu levels in breast cancer patients were markedly higher than levels in the control group. The serum Cu concentration was 1,252.20 $\mu g \bullet l^{-1}$ in the malignant group, followed by the benign group and the control group with the concentrations of 1,038.93 and 964.95 $\mu g \bullet l^{-1}$, respectively. Furthermore, a significant difference between normal and malignant tissues in the malignant group was been demonstrated with concentrations 6.13 and 11.3 $\mu g \bullet g^{-1}$, respectively. A similar result has also been observed in other types of cancer (37-39).

Recent studies have demonstrated that Cu is associated with angiogenesis (40), which is important in the proliferation, invasion and metastasis of tumor cells (41,42). Therefore, based on the biological function of Cu in tumor progression, inhibition of angiogenesis by reducing the content of Cu *in vivo* may be developed as a novel strategy in cancer therapy (4,43). Daniel *et al* (44) demonstrated that Cu complexes inhibited the activity of the 26S proteasome *in vitro* and *in vivo*. Various chelators, including 8-hydroxyquinoline, (8-OHQ), dithiocarbamate and clioquinol (CQ), may react with Cu salts and form complexes that act as potent proteasome inhibitors and apoptosis inducers in cultured human cancer cells. Further studies revealed that Cu complexes demonstrated an inhibitory effect against proteasomes *in vitro* or *in vivo* (45-47).

3. 8-OHQ and CQ

Daniel *et al* (44) synthesized and tested the anticancer activity of Cu complexes, including [Cu $(8\text{-OHQ})_2$] (8-OHQ is presented in Fig. 2). The Cu $(8\text{-OHQ})_2$ complex was revealed to be potent, transient, proteasome inhibitors capable of inducing apoptosis in human leukemia cells, but not in non-transformed, immortalized human natural cells under the same conditions. Further experimental studies demonstrated that the inhibition of CT-like proteasome activity and apoptotic induction do not result from Cu-mediated oxidative damage to proteins, but from the formation of a proteasome inhibitor inside the tumor cell (43).

CQ (5-chloro-7-iodo-8-hydroxyquinoline; Fig. 2) is an analog of 8-OHQ that has been used to treat Alzheimer's disease in preclinical and clinical trials (48-51). CQ has been demonstrated to bind Cu and form novel Cu complexes, which possess proteasome-inhibitory activities and induce apoptosis in cancer cells (44). Cu-chloride ($CuCl_2$) has the ability to directly inhibit CT-like activity of purified 20S proteasome, with a half maximal inhibitory concentration value of

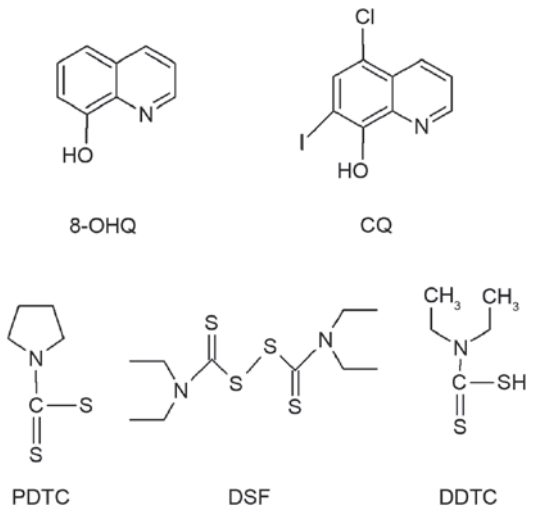

Figure 2. Chemical structures of quinoline derivatives and dithiocarbamate derivatives. 8-OHQ, 8-hydroxyquinoline; CQ, clioquinol; PDTC, pyrrolidine dithiocarbamate; DSF, disulfiram; DDTC, diethyldithiocarbamate.

3-5.3 μM (44,45); however, it does not enter cells. CQ alone did not inhibit the CT-like activity of the proteasome; however, the combination of CQ and $CuCl_2$ resulted in different effects in the same experiment and demonstrated selective inhibitory effects on CT-like activity, but not caspase- or trypsin-like activities (44,45). Subsequently, it was hypothesized that targeting highly elevated Cu in cancer cells and tissues, in conjunction with treatment with novel compounds including CQ, may result in the formation of tumor-specific proteasome inhibitors that possess potential for cancer therapy.

Chen *et al* (11) investigated the underlying molecular mechanism of CQ in human prostate cancer cells and xenografts. Inhibition of proteasomal CT-like activity, suppression of androgen receptor (AR) protein expression and induction of cell apoptosis was observed following CQ binding with Cu. CQ was subsequently administered to mice bearing C4-2B xenografts, and potently inhibited the tumor growth via *in vivo* proteasome inhibition and apoptotic induction. The study suggested that CQ is capable of targeting the tumor proteasome *in vivo* depending on the presence of Cu, and subsequently leads to the formation of an AR inhibitor and apoptosis inducer, which is responsible for the observed anti-prostate tumor effect (11,46). In 2009, to further understand the molecular mechanism of CQ and 8-OHQ-mediated antitumor activity, Barrea *et al* (47) conducted a human prostate tumor xenograft experiment. Elemental mapping and the chemical status of Cu in tumor and normal tissues collected from the same mice were then measured. The copper in normal tissue and tumor tissue existed predominantly in the form of Cu (I). Following treatment with CQ, cellular copper could interact with CQ and convert Cu (I) to Cu (II), and thus, Cu (II) content increased significantly in tumor tissue (47). This conversion of Cu (I) to Cu (II) in tumor tissue is associated with CQ-induced proteasome inhibition. Zhai *et al* (12) revealed that 8-OHQ or CQ, but not their analogs, may bind to Cu salt and transport the Cu complex into human breast cancer cells or interact with cellular Cu to form a complex and consequently result in proteasome inhibition, growth-suppression and apoptotic induction (12). However, further studies are required to verify the use of 8-OHQ or CQ in clinical trials as potential anti-cancer agents.

4. Dithiocarbamates

Dithiocarbamates, which are a class of metal chelating reagents, interact with metal ions and form metal complexes that have been applied as a class of potential agents to target the UPP in cancer treatment. Pyrrolidine dithiocarbamate (PDTC; Fig. 2) is the first member of the dithiocarbamate family, which has been revealed to bind Cu, inhibit the cancer-specific proteasome and induce cellular apoptosis in human breast and prostate cancer. However, PDTC alone failed to exhibit similar results in cultured cells (45,52).

Yu *et al* (53) and Wang *et al* (54) synthesized a series of PDTC analogues with substitutions to the pyrrolidine ring, and studied the associations between structure and activity. Following the formation of Cu complexes, it was observed that the size and polarity of the ring within PDTC affected its activity. When the pyrrolidine ring was substituted with larger and more polar groups in the analogues, the effect on proteasome-inhibitory potencies of the formed Cu complexes was significantly decreased (53). Furthermore, novel Cu (II) complexes of PDTC analogues had less aldehyde dehydrogenase (ALDH) inhibition activity and inhibited the CT-like activity of the proteasome in human breast cancer cells (54). Disulfiram (DSF; Fig. 2) was subsequently revealed to have antitumor activities when binding with Cu and forming a complex. DSF is an irreversible ALDH inhibitor, which has been clinically used for the treatment of alcoholism (55).

Chen *et al* (10) tested the effects of DSF and a DSF-Cu mixture in cultured breast cancer cells on proteasome inhibition and apoptotic induction. Cell death was observed following treatment with the DSF-Cu complex, however not with DSF alone. Conversely, following an alteration of the Cu concentration in human breast cancer cells to the Cu levels present in the patient, DSF alone resulted in a similar biological activity as observed with the DSF-Cu mixture in cultured cancer cells. These results demonstrated that once DSF entered the tumor cells, it was able to react with endogenous Cu, inhibit the proteasome and induce apoptosis in Cu-enriched MDA-MB-231 human breast cancer cells.

This previous finding was further verified following treatment of mice bearing MDA-MB-231 tumor xenografts with DSF. The results indicated that DSF inhibited the tumor growth of mice via proteasome-inhibitory activity (10). Notably, based on these preclinical studies of DSF, the use of DSF in human cancer treatment has been investigated in clinical trials. In addition, diethyldithiocarbamate (DDTC; Fig. 2) is a member of the dithiocarbamate family and a potent chelator of Cu. DDTC is a synthetic immunomodulator that has undergone clinical trials in patients with human immunodeficiency virus-1 infection; DDTC resulted in a significant delay in disease progression to acquired immune deficiency syndrome (56,57). Following binding with Cu, DDTC-Cu (II) (58) and DDTC-Cu (I) (59) complexes significantly inhibited proteasomal CT-like activity and induced apoptosis in breast, prostate and pancreatic cell lines. Furthermore, DDTC-Cu (II) complex inhibited the expression of AR protein in prostate cancer cells and estrogen receptor proteins in breast cancer cells (58). However,

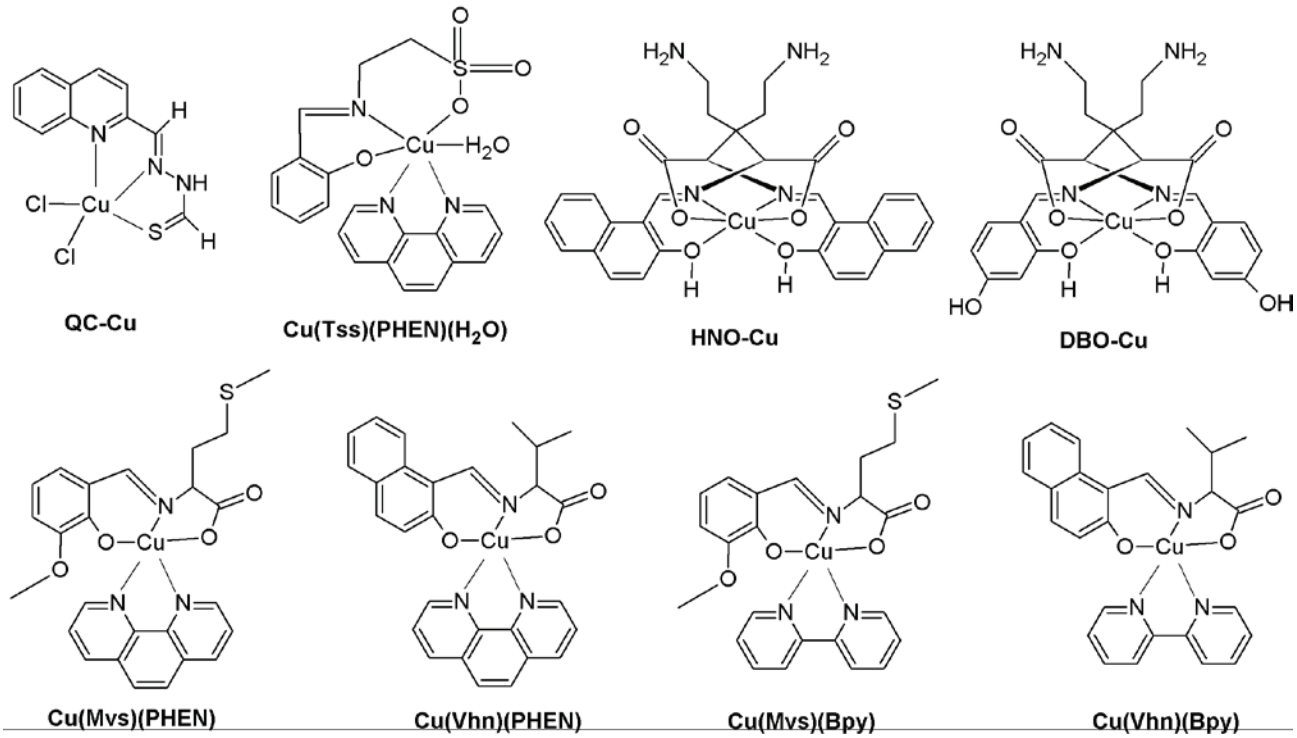

Figure 3. Chemical structures of Schiff base copper complexes: QC-Cu, Cu(Tss)(PHEN)(H_2O), HNO-Cu, DBO-Cu, Cu(Mvs)(PHEN), Cu(Vhn)(PHEN), Cu(Mvs)(Bpy), Cu(Vhn)(Bpy). QC, quinoline-2-carboxaldehyde; Cu, copper; Tss, taurine salicylic Schiff-base; HNO, 2-hydroxy-1-naphthaldehyde-L-ornithine; DBO, 2,4-dihydroxybenzaldehyde-L-ornithine; PHEN, 1,10-phenanthroline; Bpy, 2,2'-bipyridine; Mvs, L-methionine-o-vanillin Schiff base; Vhn, valine-2-hydroxy-1-naphthaldehyde Schiff base.

increased p27 and decreased nuclear factor-κB expression was detected with DDTC-Cu (I) treatment in patients with pancreatic cancer (59). The presence of Cu (I) and Cu (II) results in the formation of DDTC-Cu (I) and DDTC-Cu (II) *in vivo*, which may subsequently lead to anticancer activity. These results suggested that increased Cu in human cancer cells and tissues may be used as a novel targeting method for cancer therapy.

5. Schiff base Cu complexes

The Schiff base is a functional group, which includes a double bond between carbon and nitrogen, which may be synthesized by condensation of an aliphatic or aromatic amine and a carbonyl compound. Schiff bases and their metal complexes have been used widely in medicine, catalysis, corrosion protection and analytical chemistry, due to their physiological properties. Schiff bases and their metal complexes have previously been demonstrated to exhibit potent antibacterial and antitumor activity (60-66).

Schiff base Cu complexes have been investigated as potential anticancer drugs that target the UPP. However, the specific structure of the complex is associated with its anticancer activity. The synthesis of a series of Schiff bases of quinoline-2-carboxaldehyde (QC) and their Cu complexes (QC-Cu; Fig. 3) was previously reported (61), and these complexes were revealed to exhibit significant antiproliferative activity against PC-3 and LNCaP prostate cancer cell lines.

Notably, the biological activity and function of these complexes is affected by the nature of the side chains at position C2. An introduction of a thiocarbonyl group at the C2 position in the quinoline moiety upon Cu complexation, demonstrated the greatest cytotoxic activity. Furthermore, Schiff base Cu complexes are capable of inducing apoptosis via the inhibition of CT-like proteasome activity, however not via oxidative stress in LNCaP prostate cancer cells (61).

1,10-phenanthroline (PHEN) is an important metal chelator with a planar structure. Various metal complexes containing PHEN and Schiff bases possess anticancer activity. These include the taurine Schiff base copper complex [Cu (Tss) (PHEN) (H_2O); where Tss is taurine salicylic Schiff-base; Fig. 3], which potently inhibits the activity of the proteasome and induces apoptosis in MDA-MB-231 human breast cancer and Jurkat T leukemia cells (67). This conclusion is consistent with information presented in another report (68). A more recent study has presented the construction of four novel amino acid Schiff base Cu complexes [Cu (Mvs)(PHEN), Cu(Vhn)(PHEN), Cu(Mvs)(Bpy) and Cu(Vhn)(Bpy); where Mvs and Vhn are L-methionine-o-vanillin Schiff base and valine-2-hydroxy-1-naphthaldehyde Schiff base, respectively; Fig. 3], which contain PHEN or 2,2'-bipyridine (Bpy) as the second ligand (68). Cytotoxicity and antiproliferation studies of these Cu complexes against MDA-MB-231 or MCF-7 human breast cancer cells, and PC-3 prostate cancer cells demonstrated different effects. Notably, Cu complexes with PHEN as the second ligand were able to inhibit cell growth, proteasome activity and induce cell death; however, the compounds with Bpy as the second ligand did not. The presence of PHEN as part of the complex has therefore been demonstrated to be important in determining cytotoxic activity. The preliminary docking analysis explained why various activities are exhibited between the two substances. The complex Cu(Mvs)

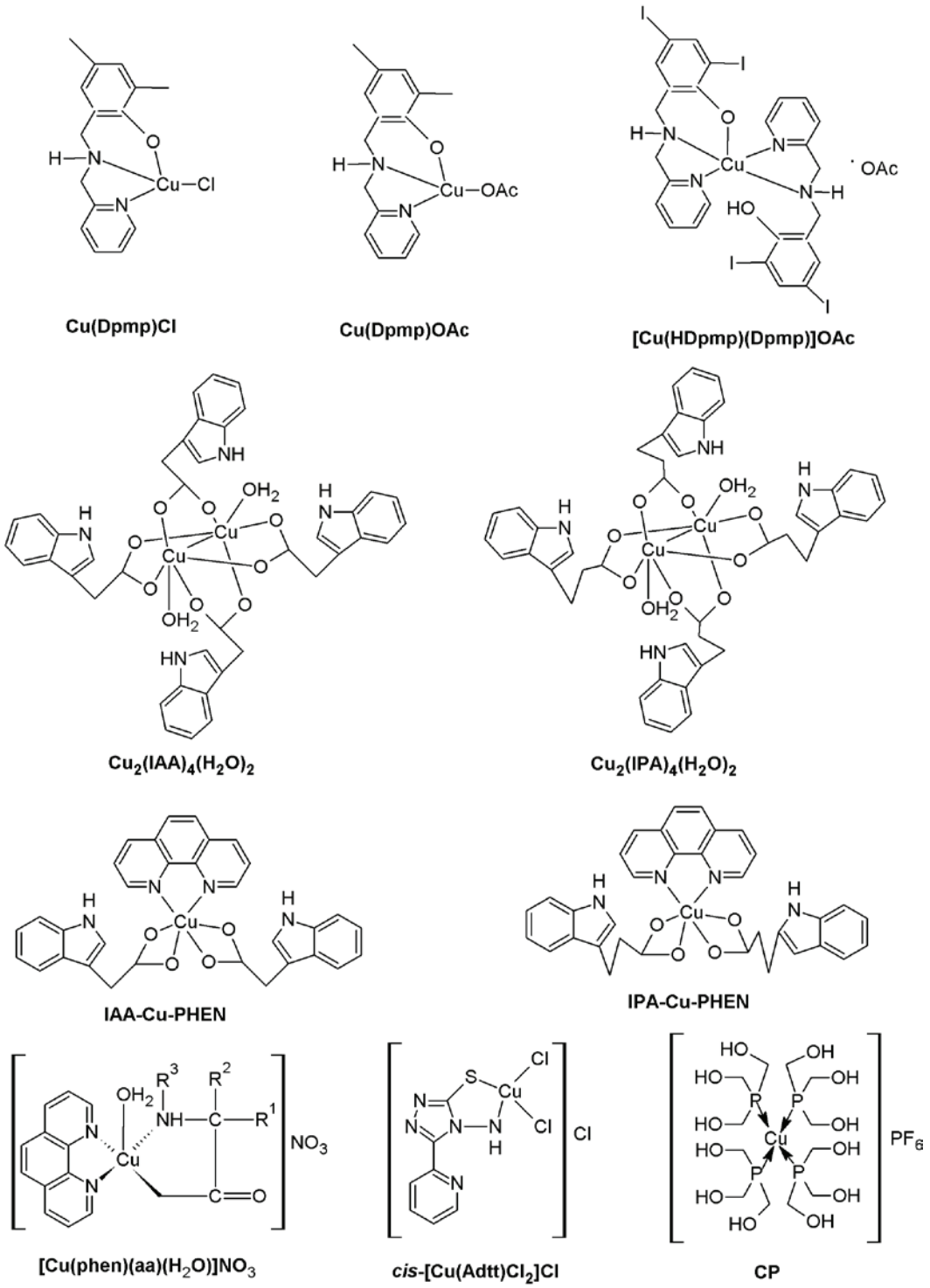

Figure 4. Chemical structures of further copper complexes: Cu(Dpmp)Cl, Cu(Dpmp)OAc, [Cu(HDpmp)(Dpmp)]OAc, $Cu(IAA)_4(H_2O)_2$, $Cu(IPA)_4(H_2O)_2$, IAA-Cu-PHEN, IPA-Cu-PHEN, $[Cu(PHEN)(aa)(H_2O)]NO_3$, *cis*-$[Cu(Adtt)Cl_2]Cl$ and CP. Cu, copper; Dpmp, 2,4-diiodo-6-((pyridine-2-ylmethylamino) methyl)phenol; IAA, 3-indole acetic acid; IPA, 3-indole propionic acid; PHEN, 1,10-phenanthroline; CP, $[Cu(thp)_4][PF_6]$; thp, tri(hydroxymethyl)phosphine; aa, methylated glycine, DL-alanine, aarcosine or 2,2-dimethyglycine; Adtt, 4-amino-1,4-dihidro-3-(2-pyridyl)-5-thioxo-1,2,4-triazole.

(PHEN) possesses important intermolecular interactions with the enzymatic pocket and fits exactly to the CT-like binding pocket, thus leading to the reversible inhibition of activity; however, Cu(Mvs)(Bpy) does not exhibit this property (68).

Schiff base Cu complexes HNO-Cu (HNO, 2-hydroxy-1-naphthaldehyde-L-ornithine; Fig. 3) and DBO-Cu (DBO, 2,4-dihydroxybenzaldehyde-L-ornithine; Fig. 3) are novel Cu-containing complexes that have been synthesized and compared for their abilities to inhibit the proliferation and induce apoptosis of MDA-MB-231 breast cancer and LNCaP human prostate cancer cells. HNO-Cu suppressed the proliferation in a dose-dependent manner, resulting in 95% inhibition of cell proliferation at 60 μM; however, DBO-Cu did not induce significant inhibition of cell proliferation in the cell lines (69). The anticancer activity is therefore dependent on the specific structure of the complex.

6. Further Cu complexes

A series of Cu complexes: [Cu(Dpmp)Cl], [Cu(Dpmp) OAc], and [Cu(HDpmp)(Dpmp)]OAc [where Dpmp is 2,4-diiodo-6-((pyridine-2-ylmethylamino)methyl)phenol; Fig. 4] have recently been reported to demonstrate anticancer activity *in vitro* against human prostate cancer and leukemia cells (70). Distinctive stoichiometries resulted in three compounds exhibiting different cytotoxicity, and the 1:1 metal-to-ligand complexes were more effective than the 1:2 species. Furthermore, it is necessary for the Cu (II) ion to interact with the ligand that acts as a carrier to enable crossing of the cell membrane. Once the compound has reached the proteasome of the tumor cell, the metal center of the complex may coordinate to identifiable amino acids, which enable Cu-N, Cu-S or Cu-O bonds to form, thus resulting in proteasome inhibition. Consequently, the active form of the Cu complex binds to the N-terminal threonine residue of the CT active center in the 20S proteasome (70).

To further investigate the association between biological activity and Cu complex structure, two types of Cu complex: Dinuclear $(Cu_2(IAA)_4(H_2O)_2$ and $Cu_2(IPA)_4(H_2O)_2$; where IAA and IPA are 3-indole acetic acid and 3-indole propionic acid, respectively; Fig. 4) and ternary (IAA-Cu-PHEN and IPA-Cu-PHEN; Fig. 4) complexes were synthesized, and the effects on cell proliferation and the ability to inhibit the activity of the proteasome were measured (71). It was observed that ternary complexes binding with PHEN as the second ligand were more efficient in carrying Cu into cancer cells compared with dinuclear complexes, and these complexes consequently resulted in deactivation of the proteasome in PC-3 human prostate cancer cells. In addition, the ternary complexes selectively inhibited the activity of the proteasome and induced apoptosis in tumor cells. The study further supported the hypothesis that carrying Cu into tumor cells to directly interact with and/or inhibit the proteasome may be applied as a potential anticancer strategy (71).

It has recently been reported that $[Cu(PHEN)(aa)(H_2O)]NO_3$ (where aa=methylated glycine, DL-alanine, arcosine or 2,2-dimethyglycine; Fig. 4) has been synthesized (72) and demonstrated the ability to induce tumor cell apoptosis via reactive oxygen species generation and proteasome inhibition (73,74). However, these particular complexes did not inhibit CT-like activity of the 20S proteasome in MDA-MB-231 breast tumor cells. However, a higher intensity and greater accumulation of ubiquitinated proteins was observed when cells were treated with the above complexes (10 μM). Therefore, proteasome inhibition by these complexes may involve the 19S regulatory cap of the 26S proteasome or the ubiquitination step (74).

It has previously been demonstrated that a Cu(II) thioxotriazole complex, *cis*-[Cu (Adtt) Cl_2]Cl [where Adtt is 4-amino-1,4-dihidro-3-(2-pyridyl)-5-thioxo-1,2,4-triazole] (75,76) and a phosphine Cu(I) complex, $[Cu(thp)_4][PF_6]$ (CP; where thp is tri (hydroxymethyl)phosphine; Fig. 4), exhibited significantly higher cytotoxic activities compared with cisplatin (77). The cytotoxic effects of *cis*-[Cu (Adtt) Cl_2]Cl and CP were associated with accumulation of ubiquitinated proteins and inhibition of the UPP in human cancer cells (76,77). The efficacy of CP was >40-fold compared with that of cisplatin in human colon carcinoma cell lines, and therefore demonstrated a notable ability to induce apoptosis. Furthermore, it was able to overcome multi-drug resistance (78). The proteasome-inhibitory activity of the majority of Cu complexes was primarily studied and optimized in accordance with the capability to specifically block the CT-like active sites. However, CP was able to inhibit all three catalytic sites of the human 26S proteasome and therefore induce paraptotic cell death (78) via the activation of endoplasmic reticulum stress signaling, which was consistent with inhibition of the UPP and induction of the unfolded protein response (76,77).

Bortolozzi *et al* (79) revealed that no mitochondrial involvement was demonstrated in the cell death process when leukemia cell lines were treated with CP. However, the activation of caspase-12, -9, -3 and -7 was observed, indicating that cell death occurred in a caspase-dependent manner. Furthermore, the 20S proteasomal chymotrypsin-like activity decreased, ubiquitinated proteins accumulated and endoplasmic reticulum stress increased markedly when CP was present. Notably, CP induced endoplasmic reticulum stress and cell apoptosis in B-acute lymphoblastic leukemia primary cells and synergistically acted with different chemotherapeutic drugs in the treatment of RS4;11 and SEM cell lines.

The combination of pyrithione (PT) and $CuCl_2$ (CuPT) may induce the accumulation of ubiquitinated proteins in cancer cells (80). Furthermore, the mechanism of CuPT may be different compared with that exhibited by the proteasome inhibitor bortezomib. CuPT may also inhibit ubiquitin c-terminal hydrolase L5 and ubiquitin specific peptidase 14 activities, which are associated with the 19S regulatory particles. Therefore, CuPT may inhibit the UPP via targeting proteasome-specific deubiquitinases and 20S proteasome peptidases; an inhibition that may have significant effects in the process of CuPT-mediated cytotoxicity (80).

7. Conclusions and perspectives

Cu is a critical component in cellular metabolism that has been demonstrated to be present at high levels in sera and tissues in various types of human cancer (36,37). It has recently been demonstrated that Cu and its coordination complexes may exhibit potential as tumor-specific proteasome inhibitors and apoptosis inducers. Two main forms of Cu chelating compound were investigated for their anticancer properties, and were revealed to exhibit proteasome-inhibitory activities. The prominent classes of metal chelating compounds, dithiocarbamates and 8-OHQs, may result in tumor proteasome inhibition and cell death by targeting elevated levels of tumor-associated Cu. However, the underlying molecular mechanisms of synthesized Cu complexes may differ depending on the mixture of Cu and small molecular ligands. The synthetic Cu complexes inhibit the proteasome by directly interacting with it, thus inhibiting its activity, or by triggering oxidation and deactivation of the cellular proteasome resulting in proteasome inactivation (41,81).

Cu-based compounds have demonstrated positive results in preclinical studies and clinical trials; however, the exact underlying molecular mechanism remains to be elucidated and

Cu-based compounds are not currently marketed as antitumor agents. Cu is essential for Cu complexes to act as proteasome inhibitors and apoptosis inducers; therefore, efforts to develop novel inhibitors based on Cu and associated ligands are of primary interest. There are at least three important strategies for the selection of ligands of Cu-based compounds during the process of novel metal-based proteasome inhibitor design. Firstly, the selection of various metal chelators, including DSF and CQ, which have been previously approved for the treatment of diseases; secondly, the synthesis of novel Cu complexes with bioactive natural compounds as ligands, including indole derivatives, pyridine derivatives and Schiff bases; finally, the synthesis of Cu complexes with compounds that are able to bind effectively to Cu, exhibiting coplanarity/planar structure.

In conclusion, safe and effective metal complexes are required for the development of novel Cu-based inhibitors. In addition, Cu complexes may be developed into potent proteasome inhibitors that target the UPP and therefore may be used to treat human cancer.

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